

Introduction to study of variegation in maize.

I. Reasons for being asked to talk on my work:

1. New genetic phenomenon discovered in maize -- not previously noted in other studies.
2. Phenomenon is probably quite general; not confined to maize.
3. Reason phenomenon not discovered earlier:
  - a). Requires a particular genetic and cytological combination of factors not readily obtainable in other material.
  - b). Requires a particular arrangement along chromosome of known "genes" affecting a particular tissue.
  - c). Requires favorable cytological conditions for detection of chromosome changes.
  - d). Type of events appeared following experiment never conducted before, utilizing a type of chromosome behavior not previously known. -- the breakage-fusion-bridge cycles.
4. Conclusions drawn from studies are essentially new and different from those previously held.
5. The newness difficult for the uninformed to evaluate without a knowledge of the experimental procedures and the types of observations made.
6. Published accounts are inadequate to understand methods used.
7. Reason for scarcity of such publications: Conclusions startled me. Therefore, necessary to make a number of different kinds of experiments in order to determine if the predictions that conclusions would indicate were followed by verification.
8. Many of the conclusions have been verified.
9. Interest expressed by some of the staff here that the experimental methods be outlined by me.
10. Therefore, Beadle and Anderson requested that I consider giving a series of talks on my material. The special course in genetics seemed to be a good place for them.
11. Intention of series of talks: (1) To develop the types of experiments conducted and the conclusions drawn from them. (2) To indicate in what way the conclusions fit with known evidence from other materials. To develop the background for the chromosome materials involved in the phenomenon.

II. The major topics:

1. The origin of many mutations.

2. The chromosomal mechanisms associated with their origin.

3. The chromosomal factors associated with controlling the time of change in genic action, the type of change in genic action, and the cells in a tissue when these changes will occur.

III. The beginnings of the first series of experiments -- why they were conducted.

1. Original purpose: To investigate the genic composition of one arm of one chromosome -- the short arm of chromosome 9 in maize. Maize has 10 chromosomes, and chromosome 9 is the second from the smallest.

2. Reason for this experiment: Previous investigations had shown:

a). Removal of minute bit of chromatin containing the normal locus of a known gene. When homozygous, reproduces the phenotypic expression of the known recessive:



Bm, Symbol for normal gene.

bm, brown mid-rib. Symbol for known recessive.

The phenotype of bm: brown color in secondary cell wall. Fades on exposure to light. Almost completely cell specific: variegation gives:

The homozygous deficiency for Bm: reproduces in all detail the phenotype produced by the known recessive, bm.

b). Conclusion: the recessive, bm, either a deficiency or an inactivation of Bm. Or, an inactivation of Bm in the cells that normally express Bm.

c). Other characters giving same indication:

Chr. 9 short arm: Yg      C      Bz      Wx

Yg to yg: yg, light colored chlorophyll in early life of leaf. Deeper at tip, lighter near base. Gradual darkening of leaf as plant ages. Rapid darkening of older leaves. Deficiency of Yg, produces the very same phenotype.

C, in combination with other known factors, produces color in the aleurone layer of the kernel. c, recessive allele, no color in the aleurone layer. Deficiency of C, produces colorless aleurone.

Bz to bz. bz, bronze, a complicated phenotype: Bz, dark color in aleurone, red or purple; red or purple anthocyanin pigment in plant. bz, known recessive, a bronze color in the aleurone and a bronze color in the plant. Also, variegation produced by loss of Bz in combination of Bz / bz, spread of Bz into bz for several cell layers. Deficiency of Bz, reproduces all phases of the recessive phenotype.

Wx to wx. Wx results in production of amylose starch in endosperm and pollen grain. wx, recessive mutant locus, results in absence of such starch, amylopectin present. Wx stains blue with iodine, wx stains red. Deficiency of Wx, reproduces the wx phenotype.

d). To return to original purpose: If the known mutants can be reproduced by deficiencies; if the known mutants reveal the genetic elements at the locus, then, removal of various bits of chromatin in the short arm of chromosome 9, one-by-one, and each separately, should reveal the genetic composition of the short arm of chromosome 9 for all cases that survive when homozygous.

e). For this test, must have a means of producing minute deficiencies in one part of the chromosome complement -- the short arm of chromosome 9 in the selected case.

f). The method worked out one paper: Required the use of the chromosomal type of breakage-fusion-bridge cycle.

(a). The essentials of this mechanism:

(1) The constitution of the gametes. (Details important; given later.)

Female

Male



(2) The fusion of broken ends in the zygote



Diagram illustrating the fusion of broken ends in the zygote.

(3) The prophase of the next mitotic division



(4) The anaphase configurations



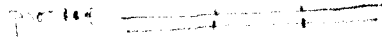
(5) The breaks in the bridges



(6) The fusions of broken ends at telophase



(7) The repetition of the cycle in the succeeding division



(b). In experiment, both broken ends are in short arm of chromosome 9.

Continuous breakage and fusion produces duplications, deficiencies, reduplications and reorientations: Examples:

(c). Observation: The b.f.b. cycle can cease in some cells. From then on, the broken ends behave as normal ends.

(g). The effects produced in the plants by the chromosome type of breakage-fusion-bridge cycles:

(1) The appearance of the seedlings

(2) The appearance of normal appearing sectors and branches.

(3) The death of the defective branches, and the survival of the normal branches.

(4). The development of the normal branches into mature plants.

V. The cytological examination of the chromosomes in the normal branches:

1). The expected types of alterations in the short arm of chromosome 9 found. Duplications of segments, reduplications of segments, rearrangements and deficiencies.

2). In addition, and this is important, a number of very unexpected types of rearrangements seen. These involved chromosome 9 and other chromosomes of the complement. Rearrangements occurred at particular places in the chromosomes in each case. These will be considered in detail later after a discussion of the organization of chromosomes has been given.

VI. The procedure used to find the expected new mutants:

1. Each branch (about 450 branches altogether) self-pollinated.

Pollen (male gametes) placed on silks of same stalk - Pollen from tassel placed on silks of same branch. The silks lead to the ovules, each ovule carries a female gamete.

2. Each ear resulting from self-pollination examined for mutations in the kernels.

3. Samples of kernels taken from each ear; seedlings grown; seedlings examined for appearance of new mutants. For reasons explained earlier, new mutants would be expected, and the locus in the chromosome complement expected to be in short arm of chromosome 9.

#### VII. The Results: General.

1. A number of new mutants appeared, as expected. In addition, a large number of new phenotypes, not at all expected.

2. The unexpected phenotypes appearing in seedlings of some cultures:

a). Example: "kernels planted from one ear; seedlings appeared; Some seedlings quite normal; others showed variegation for chlorophyll: very light green leaves with streaks of normal green.

The patterns of streaking seen in the seedlings:

b). The different types of variegation that appeared, each in a separate culture derived from kernels on a single ear:

(1). white seedling with streaks of green, pale-green; pale-green in which deep green streaks were present.

(2). Golden color of leaf. Streaks of darker color that is light green, streaks of dark green, and streaks between light green and dark green.

(3). White seedlings with spots of dark green, each surrounded by a light green "halo".

(4) Green leaves with streaks of light green.

VIII. The distribution and frequency of changes in expression. Example: light green to dark green:



IX. The appearance of the variegation in the older leaves of the plant:

1. The sectors appearing in leaves derived from seedling type-1

(A). The single sectors:

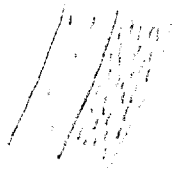
Sectors with increased frequency of green streaks  
Sectors with reduced frequency of green streaks  
Sectors with no streaks at all  
Sectors of full green color - large.

(B). The "Twin-Sectors"

Full green-no variegation: Full green, reduced frequency of streaks:



Reduced frequency of streaks - increased frequency of streaks:



2. The appearance of such types of sectoring in the many cases examined.

a). Change in the frequency of occurrence of mutation in progeny derived from a single cell.

b). Change in time of mutation -- the early mutations.

c). The relation of changes in frequency of mutations in twin sectors, and the relation of mutation in one sector to change in frequency in the sister sector.

d). The interpretation of the twin sector origins: Derived from two cells produced as the result of a single mitosis.

3. On basis of interpretation of twin sectors, the following conclusions drawn:

a). Some factor or factors present in a young cell that controls the frequency of mutation in the progeny of this cell.

b). This factor, or these factors, segregate at a mitosis such that the two sister cells differ with respect to the controlling units. This shows up as a difference in the frequency of mutation in the progeny of each. This difference is often reciprocal.

c). The mutation process also associated with a mitosis - the twin sectors showing mutant and change in frequency of mutation:

X. The conclusions from the initial observations:

- of plants that had undergone the c. b.f.b. cycle,
1. In progeny derived from self-pollinations, a large number of newly produced alterations of gene loci occurred. Many of them (over 40 found) were expressed as variegation resembling "mutable genes," in their behavior.
2. In all well examined cases, a particular type of variegation pattern present in the seedlings. The pattern -- size and frequency as well as type of mutation observed.
3. Changes occurred during the development of the plant in certain cells leading to obvious changes in the time, type, and frequency of mutation in the descendants of this cell.
4. The appearance of twin-sectors showed that something present controlling the pattern of mutation. This could be altered as a consequence of something that occurred during a mitotic cycle.
5. Also, the mutation process itself occurred as the consequence of something that occurs during a mitotic cycle.
6. Therefore: If mutation process, and if control of mutation process in a developing leaf is related to the mitotic process, it is subject to experimentation.
7. The initial purpose: What occurs during the mitotic process that is responsible for the altered genic action and the altered control of mutation in future cell generations?

XI. In next lecture, will take up the discovery of the chromosomal aspects involved in the change of phenotype - the discovery of the Ds - Ac system.